Digital Processing of Fibre Diffraction Patterns

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Introduction

Fibrous materials generally consist of particles (molecules, filaments or crystallites) which are preferentially oriented parallel to a unique axis termed the fibre axis. Diffraction patterns obtained from such materials contain information about the particles, and also about the material in which they are embedded. This matrix may consist of an amorphous phase of the same composition or may be a distinct structural entity as is the case in many biological specimens. In addition the matrix may contain liquid which also contributes to the observed diffraction pattern.

The extraction of structural information from such patterns is greatly assisted by mapping the observed intensity into reciprocal space to obtain what has been termed the *specimen intensity transform* [1]. This is a type of convolution of the orientation density function of the particles with the cylindrically averaged intensity transform of a single particle. In a perfectly oriented specimen the mapping would provide a central section through the cylindrically averaged intensity transform of the particle, but in practice the intensity at any particular point in the transform is spread out along an arc due to the imperfect alignment of the particles [2].

Reciprocal space mapping of fibre diffraction patterns has been used in studies of both simple polymers and complex biological materials to determine unit cell parameters, to collect intensity data, and to analyse systematic distortion in surface lattices [3-9]. These studies were carried out with programs tailored for specific purposes but recently a suite of programs has been developed which allow the automated extraction of unit cell parameters and structure factor data from patterns which satisfy certain criteria [10]. Many biological specimens however yield patterns that are too complex for this automated procedure to be used and still require individual treatment.

The purpose of this contribution is to direct attention to the potentials of profile analysis and of simulation in dealing with the more complex diffraction patterns, since neither have been fully exploited to date.

Profile Fitting

The advantages of profile fitting in the extraction of structure factors from single crystal intensity data are well established [11,12] and similar principles are applied in the automated procedure mentioned above [10]. If the expression used to model the profile contains parameters which are directly related the particle dimensions and the distribution of particle directions, the fitted parameters will provide important information about the physical characteristics of the specimen [13].

A major obstacle to the extraction of comprehensive structure factor data from fibre diffraction patterns is the overlapping of reflections due to the cylindrical averaging and this is compounded by the arcing due to the imperfect alignment of the particles. An approach to this problem which has considerable potential depends on the development of a realistic expression for the reflection profile in

terms of parameters directly related to the unit cell dimensions, the shape of the particle, the orientation density function, and any cumulative disorder. A detailed description of this approach and a specific example of its application to the diffraction pattern of *Bombyx Mori* silk fibroin have been given elsewhere [13].

In the case of diffraction patterns with continuous layer lines a technique has been described to deal with the smearing due to disorientation [14] and this procedure can be enhanced by profile fitting with a function which incorporates the effects of finite particle length, and the unit height and unit twist of the helix [15].

Simulation

An alternative to attempting the extraction of information directly from the specimen intensity transform is to produce a simulated diffraction pattern based on a modelling of the internal structure and spatial organisation of the particles. Visual comparison of the simulated and observed diffraction patterns, using an imaging device such as the Optronics *Photowrite,* is a valuable aid in identifying discrepancies and suggesting new approaches to modelling. Simulation is particularly useful in the study of multi-component biological materials.

Procedures for calculating the simulated diffraction patterns of fibrous assemblies of particles with 3-dimensionally crystalline structure and of particles with helical symmetry have been described [13, 14]. In the case of a 3-dimensionally crystalline particle three distinct steps are involved:

1. The 3-dimensional distribution of intensity for an infinite particle is calculated with appropriate allowance for random fluctuations in atomic positions, and the influence of solvent on the atomic scattering factors.

2. The simulated intensity distribution across a central section of the cylindrically averaged transform of a single particle is then constructed by incorporating the effects of cumulative disorder within the particle, finite particle dimensions and random azimuthal rotation about the fibre axis.

3. The intensity at each point in the simulated specimen intensity transform is then calculated by numerical integration, with appropriate weighting [2], over an arc of appropriate length in the simulated particle intensity transform.

The procedure used with helical particles is similar except that in Step 1 the cylindrically averaged intensity on each layer line is calculated at intervals of $R = 1/(2r_{max})$, where r_{max} is the radius of an exscribed cylinder enclosing the particle. Intermediate values are interpolated as required. An allowance for random azimuthal rotation is not required in Step 2 since this has already been incorporated in Step 1.

In complex biological materials the particles generally consist of helical molecules or filaments with helical symmetry. These may be packed in a 3-dimensionally crystalline array as in tendon collagen and some muscle fibres, a 2-dimensionally crystalline array as in avian and reptilian hard keratins or in quasi-

hexagonal arrays as in mammalian hard keratin. The steps outlined above can readily be modified to deal with these situations.

Most biological specimens contain appreciable quantities of water and this has a considerable effect on the intensity distribution in the low to medium angle part of the diffraction pattern. The incorporation of procedures for the simulation of this effect is essential in these materials. A simple but effective method is to incorporate the solvent correction in the atomic scattering factor [16]. The low angle region of the diffraction pattern is dominated by the shape function of the particle, which is generally modulated by an inter-particle interference function, and the electron density contrast between the particles and the surrounding medium.

Disorder in Biological Materials

Imperfections in crystalline order in biological materials often play an important role in their biological function and the elucidation of their precise nature is essential for a complete understanding of the structure-function relationship. Transform mapping and simulation provide a valuable tool for studying such disorder. Descriptions of the various types of disorder that have been observed in fibrous structures and treatments of the effects of such disorder on the diffraction pattern are widely scattered throughout the literature and it may be of value to list some of the more important sources of information.

Random displacements of the individual atoms from their idealised positions in the structure can be simulated by the inclusion of an exponential term in the atomic scattering factor [17]. The breadths of reflections and of layer lines are not affected by this type of disorder. In contrast fluctuations in lattice parameters gives rise to an increase in breadth that increases with increasing angle of diffraction [18-22] and measurements of the change in integral breadth in a related series of reflections can be used to estimate values of both the crystallite dimension in a direction perpendicular to the reflecting planes and the statistical fluctuation in the interplanar spacing. In addition to positional disorder, crystalline particles of polymeric materials may be subject to imperfections involving coordinated movements of complete chains [23,24,25] and irregularities in chain direction [25,26,27].

There is abundant evidence from studies of synthetic polypeptides that when helical molecules pack in a regular 3-dimensional array they are subject to systematic distortions due to the fact that the natural helical symmetry of the molecule is incompatible with the crystallographic symmetry [28]. This is an illustration of the concept of quasi-equivalence [29,30] which recognises the possibility that by systematically distorting a regular structure it may be possible to arrive at a structure of lower free energy. These systematic distortions generate long axial periods and layerline 'ghosts' [30-32]. In the complex assemblies encountered in biological materials disorder and systematic distortions are common [5,31-35] and a comparison of observed and simulated patterns provides a valuable means of refining model structures.

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